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NAN-190 potentiates the circadian response to light and speeds re-entrainment to advanced light cycles

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Abstract

Health problems can arise from de-synchrony between the external environment and the endogenous circadian rhythm, yet the circadian system is not able to quickly adjust to large, abrupt changes in the external daily cycle. In this study, we investigated the ability of NAN-190 to potentiate the circadian rhythm response to light as measured by phase of behavioral activity rhythms. NAN-190 (5 mg/kg, i.p.) was able to significantly potentiate the response to light both in dark-adapted and entrained hamsters. Furthermore, NAN-190 was effective even when administered up to 6 hours *after* light onset. Response to a light pulse was both greater in magnitude and involved fewer unstable transient cycles. Finally, NAN-190 was able to speed re-entrainment to a 6 h advance of the light: dark cycle by an average of 6 days when compared to vehicle-treated animals. This work suggests that compounds like NAN-190 may hold great potential as a pharmaceutical treatment for jetlag, shift work, and other circadian disorders.

Keywords

serotonin; circadian; light; rhythm; jet-lag; phase shift; transient; entrainment

Circadian rhythms are endogenously generated rhythms that coordinate processes such as hormone release, digestive enzyme secretion, and the sleep/wake cycle. In mammals, the major pacemaker is located in the suprachiasmatic nucleus (SCN) in the brain, which uses environmental cues to synchronize, or entrain, internal rhythms to the external surroundings (Rusak and Zucker, 1979; Ralph et al., 1990). Jetlag occurs when internal circadian rhythms become desynchronized from the external time. In humans, this desynchronization results in acute negative effects such as lowered performance levels on the job, difficulty sleeping at the appropriate times, and gastrointestinal problems. If frequent or prolonged, circadian desynchrony may be associated with more chronic health problems in people such as increased risk of breast cancer, cardiovascular disease, reproductive difficulties and peptic ulcers (Knutsson, 2003; Megdal et al., 2005). Studies using laboratory animals even link jetlag with increased rate of tumor growth and increased mortality in aged mice (Davidson et al., 2006; Filipski et al., 2004). Given the detrimental effects, studies have investigated several different methods to reduce circadian desynchrony. Melatonin, exposure to bright light, and gradual shifting of the sleep/wake cycle prior to jet travel in varying combinations have all been shown

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to speed re-entrainment in studies with humans (Eastman et al., 2005; Revell and Eastman, 2005; Sharkey and Eastman, 2001); however none of these methods are optimal, being inconvenient and of limited effectiveness.

The serotonergic pathway from the raphe nucleus to the SCN may be a good target for jetlag drug development. The effects of serotonin may be partially mediated by the 5HT1A receptor, which is expressed as a post-synaptic receptor in the SCN and as both a post-synaptic and an autoreceptor in the raphe nucleus. Serotonin (5HT) itself and synthetic agonists are able to phase shift the circadian rhythm during the subjective day and attenuate light-induced phase shifting during the subjective night in hamsters (Bobrzynska et al., 1996; Gannon and Millan, 2006; Horikawa et al., 2000; Rea et al., 1994; Tominaga et al., 1992). Furthermore, there have been several reports of 5HT1A antagonists potentiating the phase advance to light, suggesting that serotonergic tone on this receptor normally provides an inhibitory input to the SCN (Gannon, 2003; Gannon and Millan, 2006; Rea et al., 1995; Lall and Harrington, 2006).

In this study, we investigated the properties of NAN-190 as a pharmacological treatment for circadian desynchrony. NAN-190 acts at the 5HT1A receptor, activating autoreceptors and functionally antagonizing post-synaptic receptors (Claustre et al., 1991; Ryldelek-Fitzgerald et al., 1990). NAN-190 has previously been shown to potentiate phase shifts to light (Lall and Harrington, 2006; Rea et al., 1995). We tested NAN-190 in a series of experiments designed to evaluate the robustness of its potentiating abilities with different pre-treatments, different light pulse lengths, and different administration times with respect to the light. Finally, we evaluated its ability to speed re-entrainment to a shifted light: dark cycle as occurs in jetlag.

Materials and Methods

General Methods

Animals—Male Syrian hamsters were purchased from Charles River at 3 to 4 weeks of age and housed in translucent cages with wheels and *ad lib* access to food and water. They were stably entrained to a 14:10 light dark: cycle prior to the start of any experiment. Animals were randomly assigned to treatment groups. No animal received more than a total of 6 drug injections over the course of all 5 experiments. Animals were re-entrained to a light: dark cycle for a minimum of 10 days between experiments. Dim red light was less than 15 lux. Light pulses were administered either at circadian time (CT) 19 or zeitgeber time (ZT) 19 using 40 watt fluorescent bulbs (Sylvania, F40/DSGN50). Circadian time 12 is defined as the time of activity onset, and zeitgeber time 12 is defined as the onset of darkness in the light:dark (LD) cycle.

Data analysis and recording—Each hamster was housed with a running wheel equipped with a magnet and switch; running wheel activity was recorded and compiled into actograms by Clocklab software (Actimetrics, Evanston, IL). Daily activity onsets were determined using Clocklab, which calculates activity onsets by fitting each day's activity to a template of 6 h of inactivity followed by 6 h of activity. Onsets were edited by hand or omitted when necessary, such as when the software selected an onset that was not at the start of a significant bout of activity or data were missing due to equipment failure. Phase shifts were calculated using linear regression lines fit to the 7 onsets pretreatment and 7 onsets post-treatment, omitting the three days immediately post treatment in order to avoid transient cycles and allow the circadian rhythm to reach a steady state. Each regression line was used to predict an activity onset for the day after treatment; the difference between these two predictions was the phase shift.

Rate of re-entrainment was determined by examining the number of days needed to achieve a steady state after a shift of the light: dark cycle. A regression line was fit to the activity onsets of the last 5 days of stable entrainment 3 weeks post-treatment. This line was then extended

back to the time of the LD shift and the number of days with onsets not yet on the regression line was summed.

In order to examine transient cycles, the cycles which occur during the period in which the circadian clock is desynchronized from the environment before reaching a new steady state, we compared first day post-treatment phase changes with final phase changes for all phase shifting experiments with entrained animals. The times of activity onset for four days prior to the day of treatment were averaged; the average was then subtracted from the time of activity onset on first day post-treatment onset in order to determine the initial phase shift. This phase shift was then compared to the final phase shift determined by the regression line method described above.

Statistical analysis was performed using the SPSS 14.0 statistical package. All tests were run with an alpha level of .05. Data were examined for outliers (defined as more than two standard deviations from the mean), which were then omitted from further analyses (2 cases each in Expt 1 and 2). Groups were compared with either t-tests or ANOVA, using Bonferroni's post-hoc analysis or Tukey's HSD for multiple comparisons.

Drugs and routes of administration—NAN-190 (Sigma) was dissolved in 50% DMSO and administered via intraperitoneal (i.p.) injection at a 5 mg/kg dose. Vehicle injections consisted of 50% DMSO solution only. All injection volumes were approximately 0.5 ml and calculated based on the animal's weight as measured 2-5 days prior to treatment.

Experiment 1: Constant dark pretreatment

Methods

All hamsters (n=23) had been in constant darkness for more than 30 days at the time of treatment. On the day before treatment, activity records were examined and the projected activity onset calculated from a line fit to the past 6 days' onsets. Each animal's circadian period, or amount of time for one complete circadian cycle, was used to calculate its circadian time (CT) 19, 7 h after CT 12. CT 12 was defined as the time of the animal's activity onset. On treatment day, hamsters received injections 45 m before their projected CT 19 under dim red light. At each animal's individual CT 19, it was transported under dim red light in its cage to a separate room to receive a 5 min light pulse (approximately 3,000 lux). A clean, empty cage lid was placed over the top of the cage during the light pulse. At the end of the light pulse, the animal was transported back to the home cage room under dim red light. Activity was monitored for 10 days in constant darkness following the treatment.

Results

Our results confirmed prior studies indicating Nan-190 potentiated light-induced shifts when hamsters were housed in constant dark. Hamsters receiving NAN-190 showed a significantly greater shift to light when compared to the controls (NAN-190: 4.54 ± 1.45 h; control: 2.77 h ± 0.90 h), $t(21) = 2.64$; $p < .05$). The results from this experiment are compared to those from Experiment 2 in Figure 1, and actograms showing two individual hamsters responses are shown in Figure 2.

Experiment 2: Entrainment pretreatment

Methods

Animals (n=18) were entrained to a 14:10 light dark cycle for 3 weeks; all experimental animals displayed stable entrainment prior to receiving treatment. On the day of treatment, lights were allowed to go off at the normal time, after which they remained off until the end of data

collection (Aschoff, 1965). Treatments were given under dim red light. Nine animals received NAN-190 and 9 received vehicle injections of 50% DSMO. Animals received injections approximately 45 m before a 5 m light pulse at ZT 19 (approximately 2200 lux). The light source was the same bulb type as used in experiment 1 (Sylvania, F40/DSGN50) but four bulbs were mounted in a rack that could be placed in front of the animal cages, allowing equivalent light exposure to hamsters regardless of where theyir cage was positioned on the rack. Animals remained in their home cages in the housing room for the light pulse. After the light pulse, animals were left in constant dark for 10 days, after which they were re-entrained.

Results

This experiment demonstrated that Nan-190 is able to potentiate shifts to light even in entrained hamsters. The NAN-190-treated group showed a significantly larger phase shift (4.27 ± 0.92 h) than the vehicle-treated group (1.62 ± 0.28 h), $t(13) = 8.23$, $p < .001$). The effect was comparable to that observed in hamsters housed in constant darkness prior to the light pulse, as is shown in Figure 1. All our further experiments were conducted using hamsters entrained to a LD cycle prior to treatment.

Experiment 3: Different durations of light

Methods

Animals (n=38) were stably entrained to a 14:10 light: dark cycle at least 10 days before treatment. On the day of treatment, the lights went off at the normal time, after which they remained off until the end of data collection. Treatments were given under dim red light. Animals received injections approximately 45 m before ZT 19, at which time the light pulse commenced. Half the animals were removed from the home cage room by wheeling their housing cage rack into a dark hallway, where they remained after 15 m of light. The remainder of the animals received an additional 15 m of light during that time, for a total light pulse of 30 m duration. At the end of the light pulse, all animals returned to the housing cage room under dim red light. Activity was recorded in constant dark for 10 days.

Results

This experiment indicated that the effect of NAN-190 was more robust when combined with a 30 min light pulse as vs. a 15 min pulse (data shown in Table 1). NAN-190 significantly potentiated the advance to light in both the 15 and the 30 m light pulse conditions ($t(16) = 2.41$; $p < .05$ and $t(18) = 5.86$; $p < .01$, respectively). The two vehicle-treated groups did not significantly differ from each other ($t(17)=.299$; $p > .05$), but NAN-190 was able to significantly potentiate the response to a 30 m light pulse (138.34%) more than to a 15 m light pulse (47.27%) ($F(3, 34) = 18.201$, $p < .01$; Tukey's HSD reveals NAN-190 + 30 m light pulse significantly different from all other groups.) Based on these results, all subsequent experiments used a 30 m light pulse.

Experiment 4: Drug administration time-course

Methods

Three trials of a drug-administration time-course experiment were run. All animals (n=44) were stably entrained to a 14:10 light dark cycle for at least 2 weeks before treatment. Animals were randomly assigned to receive either NAN-190 or vehicle injection at one of four time points: either 45 m prior to the start of the light pulse; 30 m, 1 h, or 1.5 h after the start of the light pulse. In the second trial, animals (n=40) were randomly assigned to receive either NAN-190 or vehicle injection 1.5 h, 3 h, 4.5 h, or 6 h after the start of the light pulse. In the third trial, NAN-190 or vehicle injections were administered without light, at 45 m before ZT 19 (ZT 18.25), or 1.5 h, 3 h, 4.5 h, or 6 h after ZT 19 (ZT 20.5, 22, 23.5 or 1, respectively).

On the day of treatment, the lights went off at the normal time, after which they remained off until the end of data collection. Treatments were given under dim red light. Animals received a 30 m light pulse at ZT 19. At the predetermined injection times, hamsters received either the NAN-190 or vehicle injections under dim red light. At the end of the light pulse, the lights were turned off and remained off for 10 days for data collection.

Results

Surprisingly, NAN-190 was able to significantly potentiate the response to light for each treatment time, even when the drug was administered as long as 6 h following the light pulse. This can be seen in Figure 3, comparing the vehicle treated light-exposed hamsters (open bars) with the NAN-190 treated light exposed hamsters (solid filled bars). No significant differences were found between the drug-treated animals which received light ($F(6, 39) = 2.33$, $p = .05$), regardless of time of drug administration. The Student's *t* test was used for planned comparisons between vehicle- and drug-treated groups at each time point with and without light. For the animals receiving light pulses, only one significant difference was found between vehicle-treated animals at the different time points ($F(6,30)=2.68$; $p < .05$); Bonferroni's post hoc analysis revealed that hamsters treated with vehicle at 30 minutes shifted 1.04 h less than those treated at 3 h ($p = .046$). No other groups significantly differed from each other. NAN-190 caused small but significant shifts when administered without a light pulse at ZT 22 ($t(3) = -5.26$, $p<.05$), ZT 23.5 ($t(3) = -24.03$, $p<.01$) and ZT 1($t(3) = -4.47$, $p <.05$). This experiment indicated the timing of NAN-190 is much more flexible than has been reported for other compounds that influence the light-input pathways, and suggested this compound might have robust effects on re-entrainment to a shifted LD cycle.

Experiment 5: Re-entrainment to a 6 h advance of the LD cycle

Methods

Animals were re-entrained to a 14:10 light cycle (n=44). Once stable entrainment had been achieved, the light cycle was advanced six h by means of a short night (Day 1). On Day 1 of the light cycle advance, animals received an injection of either NAN-190 or vehicle either 45 m before the new lights-on time or 1 h after it. They were then left, undisturbed, to re-entrain to the new light cycle. To test if drug treatment plus one day of light exposure was sufficient for re-entrainment, a second group of animals (n= 20) received one full day of the advanced light cycle and either an injection of NAN-190 or vehicle 1 h after the start of the new light cycle. They were then placed in constant dark for 10 days.

Results

Figure 4 shows actograms from representative animals following the LD shift. The control animal required 10 days to re-entrain to the shifted LD cycle, whereas the NAN-190 treated animal was re-entrained the next day (see Figure 4). Actograms from all animals in this experiment are represented in Figure 5 where we plot the time of activity onset for each animal on each day of the experiment. It is clear from Figure 5 that the individual results shown in Figure 4 were commonly observed. Overall, NAN-190 significantly reduced the number of days needed to re-entrain to a 6 h shift of the light: dark cycle ($F(3, 36) = 31.64$, $p<.01$). Bonferroni's post hoc analysis revealed that both groups of NAN-190- treated animals re-entrained significantly faster than the vehicle- treated controls but that there was no significant effect of time of drug administration (NAN-190 45 m pretreatment: 2.4 ± 2.5 days; NAN-190 1 h post-light 1.1 ± 1.3 days; vehicle 45 m pretreatment: 7.4 ± 1.8 days; vehicle 1 h post-light: 7.8 ± 1.9). The hamsters treated with only one day of the shifted LD cycle showed results supporting the conclusion that much of the shift was accomplished within one day of the 6 h LD shift. NAN-190 treated animals shifted an average of 4.5 h whereas vehicle treated animals only shifted 2.2 h ($t(18) = -4.40$, $p<.01$).

Experiments 2-4: Transient Cycles

We noted throughout these experiments that the animals treated with NAN-190 showed few if any transient cycles. To quantify this, all phase shifts from Experiments 2-4 were examined for duration of transient cycles by comparing the percent of the final phase shift achieved on the day immediately following treatment. Percentages for each treatment group are presented in Table 1. Overall, NAN-190 treated animals achieved between 80 and 100 percent of their final phase shift, whereas vehicle treated animals achieved between 37 and 53 percent of their final shift by the first day after treatment. We show in Figure 6 that animals treated with NAN-190 achieved significantly higher percentages of their final phase shifts by the first day following treatment than did vehicle treated animals in all conditions across experiments 2-4. Vehicle-treated animals under different conditions did not differ in the percentage achieved.

Discussion

Our studies indicate that NAN-190 consistently potentiated circadian clock resetting following light exposure under various conditions. NAN-190-treated groups showed an enhanced response to light after prolonged exposure to constant dark or directly from entrainment. NAN-190's potentiating abilities may be sensitive to the duration of light exposure; however, this response may saturate around 30 m of light. Pretreatment was effective but not necessary since administration as much as 6 h following the start of the light pulse continued to be as effective. Most importantly, NAN-190 increased the rate of adjustment to a new phase. Our results demonstrate that following a 6 h advance of the light: dark (LD) cycle, an amount noted to induce maximal internal desynchrony (Leise and Siegelmann, 2006), the time needed to adjust to the new cycle diminished from 7-8 to 1-2 days as a result of a single drug administration.

As with the present findings, earlier reports noted that compounds acting at the 5HT1A receptor potentiate the response to light (Gannon, 2003; Gannon and Millan, 2006; Moriya et al., 1998; Rea et al. 1995; Lall and Harrington, 2006). These studies, however, uniformly employed an experimental design in which animals were housed in constant darkness for several days prior to treatment. Our experiments demonstrate that NAN-190 not only enhances light-induced shifts under these conditions but retains its potentiating effects when administered to hamsters currently entrained to a fixed LD cycle. This is an important distinction as it more readily allows extrapolation to everyday living (albeit in a nocturnal species). Shifts in the laboratory LD cycle can then be viewed as shifts in the rest-activity cycles that occur in transmeridian travel or as a result of lifestyle choices.

Transient cycles are those that are generated during the period that the circadian clock is desynchronized from the environment before reaching its re-synchronized steady state (Pittendrigh et al., 1958). Following a phase shift, peripheral oscillators in various internal organs also become desynchronized, a condition thought to underlie the symptoms of jet-lag (Yamazaki et al., 2000). On a human scale, the transient cycles represent the days of gradual re-synchronization, in which physical symptoms such as tiredness and gastrointestinal upset occur (Revell and Eastman, 2005). Sildenafil, a potent and selective inhibitor of phosphodiesterase 5, has recently been shown to increase the rate of re-entrainment of hamsters following a 6 h advance of the LD cycle, decreasing the number of days required for re-entrainment by up to 50% (Agostino et al., 2007). However, sildenafil did not appear to alter the number of transient cycles following a single light pulse-induced phase shift, whereas in our experiments NAN-190 sped the rate of resetting such that fewer transient cycles occurred. The latter was observed both in the single light pulse and re-entrainment paradigms; earlier studies reported similar abrupt phase shifts with NAN-190 (Rea et al., 1995) and with BMY 7378 and S15535, compounds that also act as 5HT1A partial agonists (Gannon, 2003).

While we have demonstrated the ability of NAN-190 to potentiate the light-induced advance in wheel-running activity, we do not know whether the compound would yield potentiated shifts in peripheral oscillators as they may occur in (e.g.) the liver or lungs. MKC-242, a selective 5-HT1A partial agonist, is able to potentiate light-induced shifts in mice (Moriya et al., 1998; Takahashi et al., 2002); consequently, there is good reason to believe NAN-190 will also, offering the possibility of measuring similar effects on peripheral oscillators using luciferase reporter animals (e.g. Yoo et al., 2004). The relationship between the central and the peripheral oscillators may be just as important to human functioning as the relationship which ties SCN timekeeping to the solar day; it may also be directly related to the symptoms of shift lag during transient cycles (Shibata, 2007; Yamazaki et al., 2000).

NAN-190 has high affinity for 5HT1A receptor sites, where it acts as a partial agonist (Claustre et al., 1991; Rydelek-Fitzgerald et al., 1990). On raphe neurons, limited activation of cell body autoreceptors is sufficient to diminish firing frequency, as a result of extensive receptor reserve (Fornal et al., 1994). On postsynaptic sites where receptor reserve might be less extensive, the same degree of partial agonist receptor activation can antagonize the action of more robust agonists; this has been shown with 8-OH-DPAT (Claustre et al., 1991). The net effect of diminished cell firing (and consequently diminished 5-HT release) and functional postsynaptic receptor antagonism is a reduction in serotonergic tone to the SCN. The same is true for MKC-242, although this compound may display greater agonist properties in the agonist-partial agonist-antagonist continuum (Matsuda et al., 1995). Interestingly, both compounds also share the ability to block alpha-1 adrenergic receptors; at the level of the raphe nuclei, this action too can reduce cell firing by removing excitatory noradrengic tone (Baraban and Aghajanian, 1980). Removing the serotonergic tone from the SCN may account for the greater responsiveness to light.

NAN-190 is also able to induce small phase advances without light in the late night or early morning, but not earlier, at ZT 19. However, the small shifts (approximately 1 h) seen with NAN-190 alone are not sufficient to explain the large shifts (4.5-6 h) seen when NAN-190 administration follows a light pulse, either by themselves or through additive phase shifts.

NAN-190's cellular mechanisms are as of yet unclear. MKC-242 has been shown to prolong the light-induced increases in *both per1* and *per2* mRNA (Takahashi et al., 2002), which normally decay within a few hours (Best et al., 1999; Khammanivong and Nelson, 2000). Since both *per* mRNA and Per protein levels are correlated with behavioral phase shifting (Best et al., 1999; Masubuchi et al., 2005), it is possible that the greater phase advance to light observed with MKC-242 and NAN-190 may be due to their extended elevation of *per*, and thus Per protein. Such might be accomplished by allowing enhanced Per production from the light-induced *per* mRNA or by affecting the stability of Per protein (which is known to alter phase-shift magnitude) (Gallego et al., 2006; Jakubcakova et al., 2007; Lee et al., 2007; Masubuchi et al., 2005). Naturally, there are many other potential targets in addition to *per* and Per protein and at this point in time, they cannot be ruled out. However, it is clear that the compound is not altering cellular events coincident with light; NAN-190 is effective when given between 1 and 6 hours after the light pulse, strongly supporting the idea that the interaction occurs later in the intracellular cascade.

Of the two short light pulse durations employed, NAN-190 yielded greater potentiation to the longer pulse with average phase advances approximately 4 hours in length. Earlier studies suggested that the hamster circadian system is normally only able to phase shift a maximum of approximately 2 hours per day (Nelson and Takahashi, 1999), an idea supported by our vehicle-treated controls. In other words, after a certain quantity of light, achieved either through intensity or duration, the system saturates and is unable to shift more (Khammanivong and Nelson, 2001; Nelson and Takahashi, 1999). The saturation point may occur around 30 m of

light with NAN-190; a full day of a shifted light:dark cycle did not yield a larger average shift than the 30 m pulse did.

Finally, NAN-190 or similar compounds may have clinical application. As noted earlier, current jetlag treatments consist of exposure to light pulses, shifting the sleep-wake cycle gradually prior to travel, and taking melatonin before the altered bedtime (Eastman et al., 2005; Revell and Eastman, 2005; Sharkey and Eastman, 2001). Each of these methods is less than optimally effective and undoubtedly inconvenient for the subject. NAN-190 (or compounds like it) offers the promise of much quicker and more effective clock resetting, with the convenience of one-time dosing on the day of the shifted schedule. Proof might come by selecting agents for study that are currently marketed. For example, buspirone, or Buspar, has been shown to accelerate the rate of re-entrainment in phase-advanced hamsters (Tominaga et al., 1992). Our results raise the possibility that this anxiolytic/antidepressant and other 5HT1A receptor partial agonists might be an untapped source for the relief of jet-lag and associated circadian rhythm disorders.

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Abbreviations

5HT
: 5-hydroxytrptamine, serotonin

CT
: circadian time

SCN
: suprachiasmatic nuleus

LD
: light;dark

ZT
: zeitgeber time

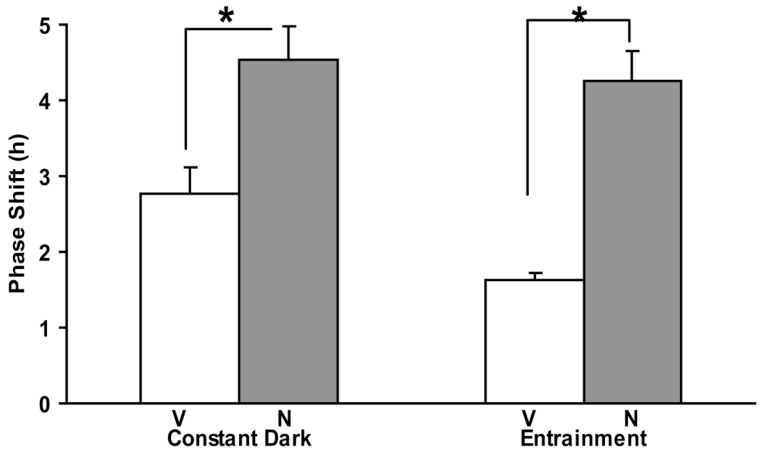

Figure 1.

Mean phase shift for light alone, vehicle (V)- and drug (N)- treated animals both from constant dark and from entrainment. NAN-190 treated animals show a significantly greater phase shift than controls in both paradigms. Asterisks indicate significance ($p < .05$); error bars represent SEM.

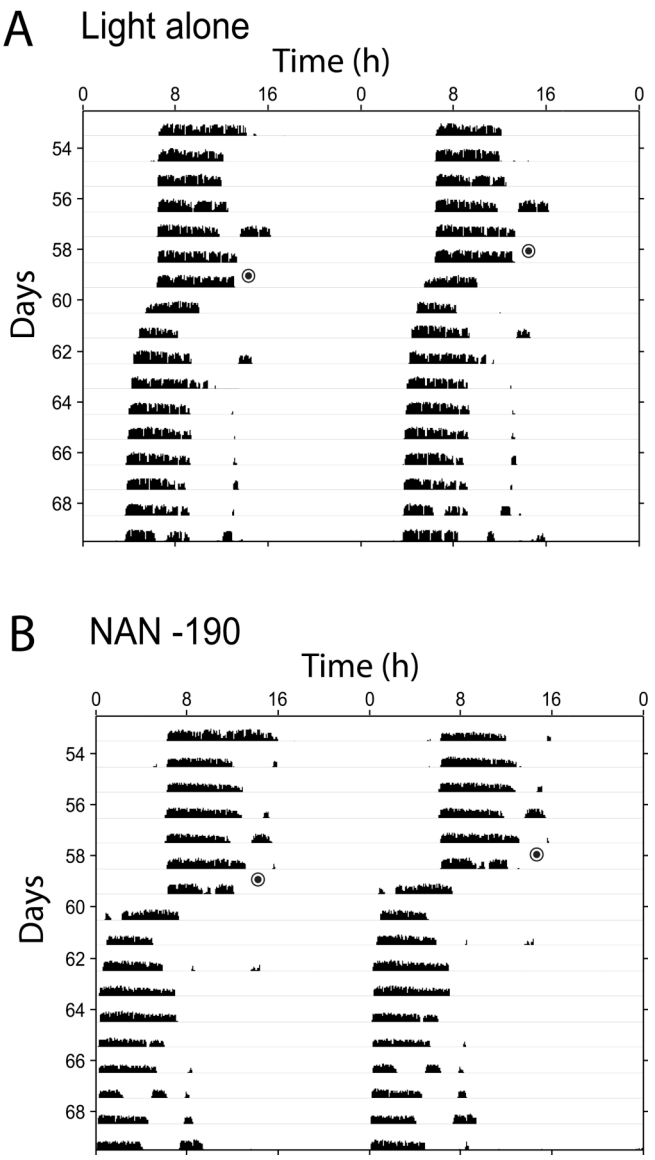

Figure 2.

Representative activity records from two animals from experiment 1. Bull's-eye indicates time of treatment A: An animal that received the light pulse only, showing a moderate shift with three days of transient cycles before steady state is achieved. B: A NAN-190 treated animal, showing a large shift with no transient cycles.

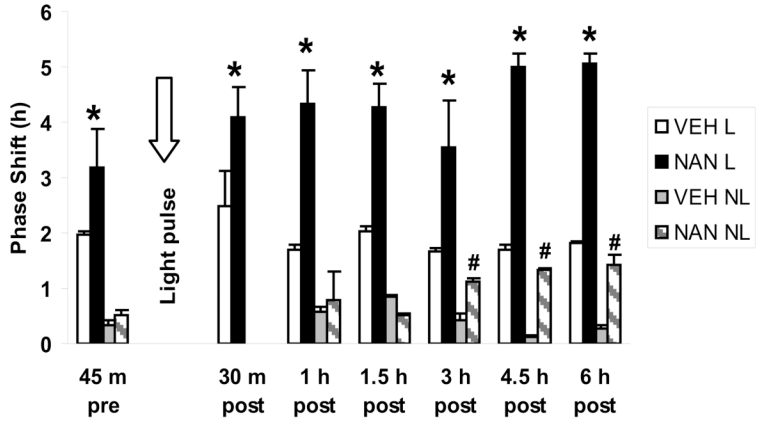

Figure 3.

An administration time course showing the mean phase shifts of animals treated with vehicle (white bars) or NAN-190 (black bars) 45 minutes before the light pulse onset or 30 minutes, 1, 1.5, 3, 4.5, or 6 hours after, with the arrow indicating relative placement of the light pulse. NAN-190 was able to significantly potentiate the response to light at all time points (indicated by asterisks.) Gray and striped bars indicate vehicle-and NAN-190-treated (respectively) animals with no light exposure. NAN-190 was able to induce a small, significant shift on its own when administered at later time points (indicated by #; $p < .05$). Error bars represent SEM.

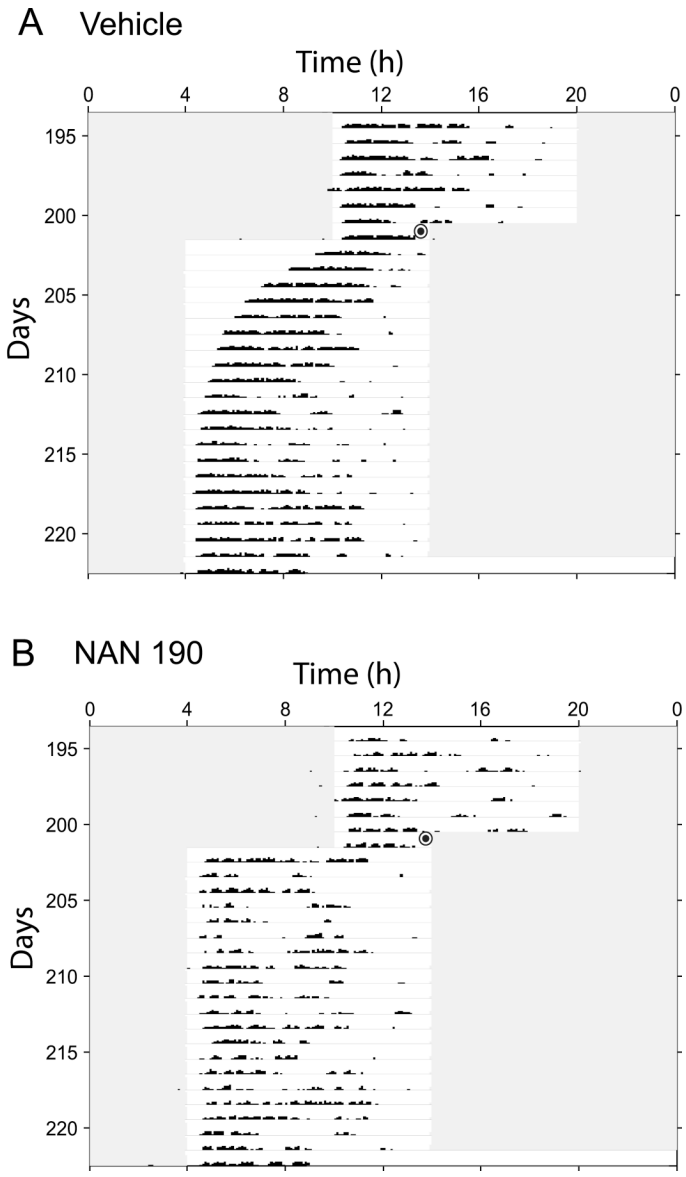

Figure 4.

Representative activity records from two animals during the re-entrainment period of experiment 5. Bull's-eye indicates time of treatment. Both animals received injections 45 m before the new lights on. A: A vehicle treated animal, which took 10 days to re-entrain. B: A NAN-190 treated animal, which was re-entrained the next day. Again, the NAN-190 treated animal displays many fewer transient cycles and achieves a steady entrainment significantly faster than the vehicle treated animal.

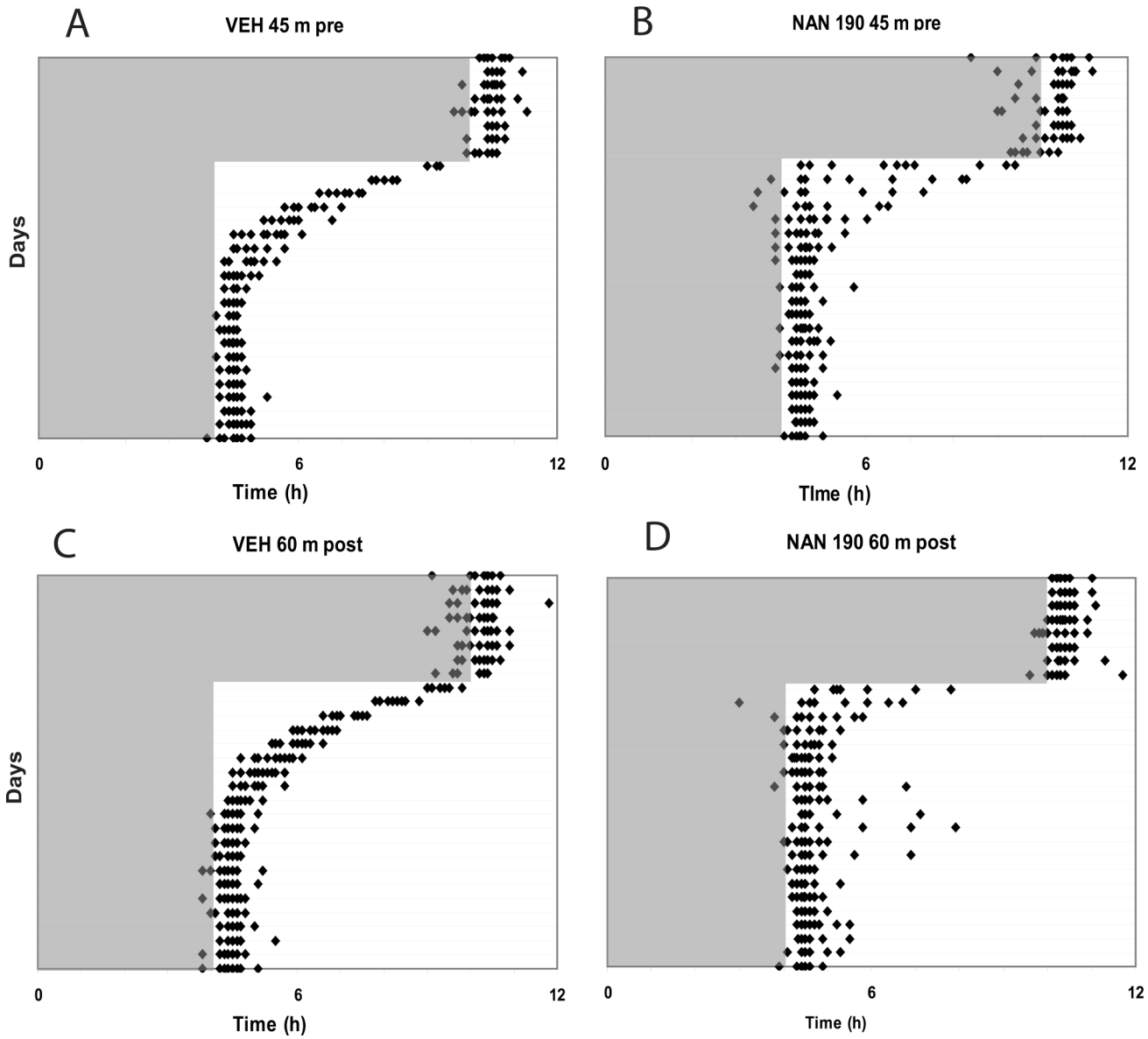

Figure 5.

Plots of the onsets of vehicle- and drug-treated animals during experiment 5. Each point represents the onset of 1 day; successive days are plotted below each other. A, C: Vehicle-treated animals at 45 minutes before and 60 minutes after new lights on. B, D: NAN-190-treated animals 45 minutes before and 60 minutes after new lights on. Vehicle treated animals show a more gradual curve to re-entrain to the new light cycle than do drug-treated animals, regardless of time of drug administration. Shading indicates time when the lights are on.

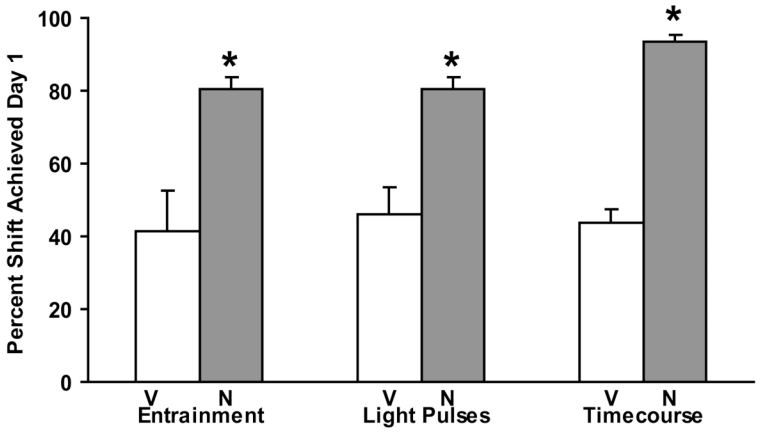

Figure 6.

Transient cycles as measured by the percent of the final shift achieved on day 1 following treatment for vehicle- (V) and NAN-190-treated (N) animals in the experiments 2 ("Entrainment"), 3 ("Light pulses"), and 4 ("Time course"). NAN-190 treated animals consistently achieve twice as much of their shift as did vehicle treated animals. Error bars indicate SEM. Asterisks indicate statistical significance ($p < .05$).

Table 1

The experimental design, mean phase shifts, standard deviations, group sizes, and transient measures for all groups across experiments 1-5. “N/C” indicates groups for which this measure was not calculated.

Experiment	Constant Dark Pretreatment	Time of drug relative to light	Light pulse duration (m)	Drug	Shift ± St. Dev (hrs)	% final shift achieved Day 1
1	Yes	45 m pre	5	VEH	2.77 ± 0.90 (14)	54
				NAN	4.54 ± 1.45 (7)	86
2	No	45 m pre	5	VEH	1.62 ± 0.28 (9)	41
				NAN	3.23 ± 1.74 (9)	80
3	No	45 m pre	15	VEH	1.74 ± 0.48 (9)	38
				NAN	2.49 ± 0.81 (9)	72
			30	VEH	1.66 ± 0.55 (10)	54
				NAN	3.97 ± 1.11 (10)	88
4	No	45 m pre	30	VEH	1.96 ± 0.16 (4)	44
				NAN	3.17 ± 0.70 (7)	86
			0	VEH	0.34 ± 0.13 (2)	N/C
				NAN	0.50 ± 0.19 (3)	N/C
		30 m post	30	VEH	2.48 ± 1.29 (4)	45
				NAN	4.09 ± 0.56 (7)	95
		1 h post	30	VEH	1.71 ± 0.16 (4)	47
				NAN	4.32 ± 0.63 (7)	100
			0	VEH	0.58 ± 0.14 (2)	N/C
				NAN	0.79 ± 0.90 (3)	N/C
		1.5 h post	30	VEH	1.97 ± 0.46 (4)	38
				NAN	4.25 ± 0.61 (7)	93
			0	VEH	0.85 ± 0.04 (2)	N/C
				NAN	0.52 ± 0.07 (3)	N/C
		3 h post	30	VEH	1.66 ± 0.16 (5)	N/C
				NAN	3.55 ± 1.89 (5)	N/C
			0	VEH	0.41 ± 0.18 (2)	N/C
				NAN	1.12 ± 0.13 (3)	N/C
		4.5 h post	30	VEH	1.70 ± 0.20 (5)	N/C

Experiment	Constant Dark Pretreatment	Time of drug relative to light	Light pulse duration (m)	Drug	Shift ± St. Dev (hrs)	% final shift achieved Day 1
				NAN	5.00 ± 0.57 (5)	N/C
				VEH	0.13 ± 0.04 (2)	N/C
			0	NAN	1.33 ± 0.06 (3)	N/C
				VEH	1.82 ± 0.05 (5)	N/C
			30	NAN	5.07 ± 0.33 (5)	N/C
				VEH	0.26 ± 0.11 (2)	N/C
		6 h post	0	NAN	1.41 ± 0.34 (3)	N/C
				VEH	2.20 ± 0.43 (10)	N/C
5	No	1 h post	14 h	NAN	4.46 ± 1.57 (10)	N/C